THE INFLUENCE OF DIETARY FAT ON THE GLYCERIDE STRUCTURE OF ANIMAL FAT

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NOTE

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In recent years there has been increasing interest in the glyceride structure of animal depot fats. Unfortunately, there is a pronounced tendency to develop unified theories to account for whatever structures are found, whether in plants or animals. Thus, Hilditch developed the concept of "even distribution", although, as he has recently emphasized (1,2) he did not propose it as a "rigid" rule. Nevertheless, Hilditch has attempted to apply the "even rule" to animal fat, explaining the deviation from it in "stearic rich" depot fats by the concept of "biohydrogenation" in the tissues (2,3,4).

Kartha has rejected the "even distribution" rule and substituted a unified rule which he believes can be used for calculating the glyceride structure of any natural fat (5,6,7). Kartha's concept is that all lipases have equal affinity for all fatty acids, and vice versa. He proposes the theory that glycerides are synthesized with random distribution of the fatty acids until any further production of saturated triglycerides would produce a solid fat. Subsequently, saturated acids are distributed without the production of saturated triglycerides.

Morris and Mattil (9) pointed out several years ago that "The fat of the larger land animals, being derived from both animal and plant sources, would be expected to vary in glyceride structure, depending upon the diet of the animal and the extent to whichthe fat is absorbed as such or altered by interesterification, ester exchange and hydrolysis and resynthesis during metabolism". Nevertheless, Kartha, in a very recent contribution to this subject stated that "in adipose tissues as well as in mammary glands of animals, fat can be deposited from ingested foods without affecting the normal glyceride type distribution" (7).

Deuel has reviewed the various attempts, except Hartha's, to explain the distribution of fatty acids in natural glycerides (8).

Unfortunately, few, if any, studies have been made of the structure of the glycerides produced by animals on fat free diets. The theories developed have been highly speculative, based on data of glycerides obtained at random.

The present study is an effort to determine experimentally the divergence of endogenous animal glycerides from the "random" or "even" type distribution and the effect on it of exogenous fat.

EXPERIMENTAL

Groups of albino rats and New Hampshire-Delaware Cross chicks were reared on an essentially fat-free ration (Table 1). The neutral fats extracted from these animals were then fed at the 20% level to second groups receiving the same basal ration. Under these conditions the glycerides fed are identical to body glycerides.

Third groups were fed 20% cottonseed oil in the basal ration since this fat conforms very closely to the "even" type distribution (10). A fourth group of chicks received 10% cottonseed oil.

Rats

Low fat ration: Several gravid females were placed on the low fat ration until after the young were weaned. The young were then separated according to sex and maintained on the fat free ration until the females weighed between 200 and 250 gms and the males between 400 and 450 gms.

The animals were sacrificed, ground in a meat chopper, and the neutral fat extracted and analyzed as described below.

Rat fat ration: A gravid female was maintained on the low fat ration until her young were weaned. Six of the young were continued on the same ration until 12 weeks of age. They were then placed in individual metabolism cages and allowed from 3 to 5 gms of feed per day for a week during which time they lost between 17% and 20% of their weight.

Fat, extracted from the group on fat-free ration, was substituted at the 20% level for sucrose and the fat ration offered ad libitum for 5 weeks, during which time they gained approximately 75 gms.

The animals were then sacrificed and the fat extracted.

Cottonseed oil ration: Four weanling females and 3 males were placed on the basal ration plus 20% cottonseed oil for six months. They were then sacrificed and the fat extracted.

Chicks

Low fat ration: Sixty straight-run New Hampshire-Delaware Cross chicks were maintained on the low fat ration for 12 weeks. They were sacrificed and the fat extracted.

Chicken fat ration: Six New Hampshire-Delaware Cross chicks were fed the low fat ration ad libitum for eight weeks and then allowed only 25 gms of feed per day for two weeks, during which time they lost approximately 40 gms of weight. They were then fed ad libitum the basal ration plus 20% of the fat extracted from the chicks on low fat ration for three weeks.

At the end of the fasting period they weighed an average of 905 grams which increased to 1832 gms during the 3 weeks on chick fat ration.

Cottonseed oil rations: Four chicks of the same breed were fed the basal ration plus 10% cottonseed oil and 8 were fed 20% cottonseed oil for 6 weeks. They were sacrificed and the fat extracted.

Analytical Procedures

Extraction: The intestines were removed and cleaned, and the entire animals ground in a meat chopper. The tissue was extracted with 3:1 alcohol-ether and washed with ether until the washings contained very little fat. The alcohol and ether were evaporated under vacuum, and the fat extracted from the water with petroleum ether. This was evaporated and the fat dissolved in warm acetone containing $MgCl_2$.

Samples taken for analyses were passed through a silicic acid column to remove traces of phospholipide (11).

Fatty Acid Composition: The polyunsaturated, oleic, and saturated fatty acids were determined spectrophotometrically (12).

Saturated triglycerides: The saturated triglycerides were determined by means of isotope dilution (13). In outline, 100 mg. of labeled tripalmitin, prepared with labeled palmitic acid, was added to 10 gm. of fat. The mixture was dissolved in 10 volumes of dry acetone and held at 8°C for 8 hours. The crystals were filtered, washed with cold acetone (8°C), redissolved in an estimated 5 volumns of acetone and held for 2 hours at 25°C. Crystallization at 25°C was repeated 3 more times. The crystallized saturated triglycerides were freed of acetone and the activity determined by direct counting technique.

After determination of activity the iodine number was assayed. With few exceptions the iodine value was not over 1.5, so that all unsaturation could be calculated as dipalmitylmonoolein. The precentage of saturated triglycerides, as determined by isotope dilution, was corrected accordingly.

$$T_s = \frac{W_k\left(\frac{A_k}{A_{uk}} - 1\right) - W_o}{W_s} \times 100$$

T_s = % saturated triglycerides, W_k = weight of labeled tripalmitin added, W_o = weight of monoolein calculated from the iodine number, W_s = weight of the sample, A_k = activity of the tripalmitin, and Auk = activity of the isolated triglycerides.

By this procedure cottonseed oil contains no saturated triglycerides. In recovery tests between 95% and 100% of synthetic tripalmitin added to cottonseed oil could be accounted for.

RESULTS

The composition of the rat and chick fats are given in Tables II and III respectively. The results are striking. The rats on the low fat diet produced a fat which conformed closely to that expected by random distribution. The addition of rat fat to the low fat diet reduced this to about 80% of the expected value. The fat produced by including 20% cottonseed oil in the ration reduced the saturated triglycerides to 76% of the value expected by random distribution.

The percentage of saturated triglycerides in chicken fat, resulting from the ingestion of either the low fat or chicken fat rations, are approximately 150% of that expected from random distribution. As unexpected and surprising as this may be, the results after feeding cottonseed oil are even more unexpected. After feeding 10% cottonseed oil the percentage of saturated fatty acids increased and the percentage of saturated triglycerides increased proportionately so that the latter remained at 50% above the expected level. After ingesting 20% cottonseed oil, however, the percentage of saturated acids fell, as one should expect, but the saturated glycerides, although they were reduced in percentage of total fat, were present in 235% of the value expected by random distribution.

DISCUSSION

It was decided to evaluate the degree of divergence from "even" and "random" type distribution by a comparison of the percentage of saturated triglyceride in the sample with the theoretical values calculated from the percentage of saturated fatty acids. Hilditch has pointed out (14) that "tendency towards coincidence" between the calculated and actual content of trisaturated triglyceride does not necessarily demonstrate that a fat mixture conforms to the "random" type distribution when the percentage of saturated fatty acid is very low or very high. Between saturated fatty acids values of 30 and 70 percent, however, comparison of the actual percentage of saturated triglycerides with the calculated values for "even" or "random" type distribution does give a measure of the extent of divergence of the fat from the two hypothetical types.

Rats

Examination of Table II shows that on a low fat diet the glycerides structure of rat fat conforms closely to that expected for "random" distribution. If it conformed strictly to "even" distribution there would be no saturated triglycerides.

After the ingestion of either rat fat or cottonseed oil, however, the percentage of saturated triglycerides was only between 75% and 80% of that expected by random distribution. If one were confronted with the cottonseed oil values only, he might be inclined to interpret the results as indicating that the "even" type oil was absorbed unhydrolyzed. Consideration of the value after rat fat feeding makes this interpretation untenable since in this case a randomly distributed fat was ingested. It would appear that the mechanism of glyceride resynthesis in the intestinal mucosa somehow tends

to an "even" type distribution after hydrolysis of the fat to monoglycerides or beyond (15).

Although Hartha has assumed that lipases are unselective in their ation, such is certainly not the case. The specificities of lipases have been recently reviewed (16). In addition, recent studies on in vivo oxidation of fatty acids show clearly that there are at least three enzymes that activate the esterification of saturated fatty acids with coenzyme A, depending on the molecular weight of the acid (17). That the esterifying enzymes of triglyceride synthesis have optimum activity for saturated acids is probable (18,20).

The probability that the synthesizing enzymes have different rates of reaction on different fatty acids could explain the tendency to "even" distribution of fatty acids in triglycerides resynthesized during intestinal absorption. Thus, if the esterifying enzyme concerned has a higher affinity for either saturated or unsaturated acids, there will be a higher rate of esterification of that acid. In addition, since it has been demonstrated clearly that lipases hydrolyze one acid from a triglyceride more readily than two, and two more readily than three (19), the converse may also be true. That is, monoglycerides might be synthesized more easily than diglycerides and triglycerides the least readily. In summary, an enzyme with a higher affinity for palmitic acid than oleic, will first prepare monopalmitin, then palmitylolein and finally the triglyceride with evenly distributed fatty acids.

The apparent "random" type distribution of the endogenous glycerides produced on a fat free diet is more difficult to explain. A factor which might influence randomization of endogenous fat is the dynamic state of

tissue glycerides. Unpublished evidence obtained in this laboratory with glycerides labeled in both the glycerol and fatty acid fractions shows that the glycerol moiety disappears from tissue fat more rapidly than the fatty acids. This indicates a constant ester exchange. It is also possible that, in tissues, there are many different fat splitting and fat synthesizing enzymes with different reaction rates and specificities, a condition which would also tend to randomization.

Chicks

The results of the studies with chicks are given in Table III. The differences between the chick and rat are striking. Thus, on the low fat regimen, after 20% chick fat and after 10% cottonseed oil, the level of saturated triglycerides is 150% above that expected for "random" distribution. After 20% cottonseed oil ingestion the saturated triglycerides were 235% of that expected of "random" distribution.

Whereas, in the case of the rats, it was necessary to explain a tendency to even distribution of ingested fat, in the case of the chick one must explain a tendency to a directed esterification to form simple triglycerides. Possibly the elevated temperature of the bird can explain the difference. It has been demonstrated that fatty acid activating enzymes have a higher affinity for saturated acids (18,20). The increased rate of reaction at the higher temperature of the bird may increase the speed of esterification on the 2 and 3 positions of the glycerol sufficiently to account for the small increase in amounts of trisaturated glyceride above that expected by random distribution.

SUMMARY AND CONCLUSIONS

In order to determine the glyceride structure of a representative mammal and bird, rats and chicks were raised on an essentially fat-free ration and the percentage of saturated triglycerides in their neutral fat determined by an isotope dilution procedure.

In order to determine the influence of ingested fat, second groups were fed the fat extracted from the animals in the first group, at the 20% level. Third groups were fed cottonseed oil, which has "even" distribution of its fatty acid.

It was found that:

1. The glyceride structure of endogenous rat fat conforms to the "random" type distribution.

2. Ingested fat appears to be digested and resynthesized by the rat according to "even" type distribution, or, at least, in a manner which tends to distribute the fatty acids.

3. Chicks tend to produce simple or "mono-acid" glycerides (8) in which the percentage of trisaturated glycerides is higher than expected for random distribution.

4. It is suggested that the findings can be explained by a selective affinity of the esterifying enzyme system for saturated acids and for the 1-position on the glyceride molecule. In the case of the bird, its higher body temperature may increase the speed of the reaction on the 2- and 3-positions of the glycerol sufficiently to account for an increase in trisaturated glycerides above that required by random distribution.

Table I

Basal Ration

	%		%
Soybean protein	25.0	Methionine	0.8
Sucrose	58.6	Glycine	0.5
Salts	6.0	Choline	0.2
Dried whey	5.0	Inositol	0.2
Liver L[2]	4.0	Cottonseed oil	0.1
		Mixed tocopherols	0.1

	mg/kg		mg/kg
Niacin	100	Pyridoxine	8
P-aminobenzoic acid	100	Thiamine	6
Ca-pantothenate	40	Folic Acid	4
Carotene	33	Menadione	0.5
Riboflavin	12	Biotin	0.2

Table II

Fatty Acid and Saturated Triglyceride Composition of Rat Fat on Rations Containing 0.1% and 20% Cottonseed Oil and 20% Rat Fat[1]

Ration	No. rats	Fatty Acids				Saturated triglycerides		
		Oleic	Linoleic	Linolenic	Saturated	Calculated	Found	% of Calculated
		%	%	%	%	%	%	
0.1% CSO	14	70	2.7	0.3	26	1.76	1.89	107
20% CSO	7	49	31.0	0.4	20	0.80	0.69	86
20% Rat Fat[1]	6	71	4.3	0.2	25	1.56	1.23	79

[1]This fat was extracted from rats fed 0.1% cottonseed oil.

[2]Assuming random distribution. The values are approximations assuming the same molecular weight of all acids: % saturated triglycerids = $\left(\frac{\%\ \text{saturated acids}}{100}\right)$

Table III

Fatty Acid and Saturated Triglyceride Composition of Chick Fat on Rations Containing 0.1%, 10% and 20% Cottonseed Oil and 20% Chick Fat[1]

Ration	Expt. No.	No. Chicks	Fatty Acids				Saturated Triglycerides		
			Oleic	Linoleic	Linolenic	Saturated	Calculated[2]	Found	
			%	%	%	%	%	%	% of Calc.
0.1% CSO	1	6	66.3	2.7	0.3	29.2	2.51	3.64	
0.1% CSO	2	6	67.0	2.7	0.4	29.4	2.56	3.42	
0.1% CSO	3	48	68.0	2.8	0.3	28.6	2.35	3.49	
Average			67.1	2.7	0.3	29.1	2.44	3.52	144
10% CSO	1	1	36.1	28.4	0.3	34.5	4.15	6.04	
10% CSO	2	1	45.2	24.6	0.0	30.0	2.68	6.54	
10% CSO	3	2	53.0	21.0	0.3	34.4	4.08	4.08	
Average			44.8	24.7	0.2	33.0	3.59	5.55	155
20% CSO	2	8	48.2	30.0	0.0	21.3	0.93	2.19	235
20% chick fat[1]		1	70.5	1.1	0.3	27.6	2.09	3.31	
		1	72.2	1.2	0.0	25.8	1.72	2.99	
		1	70.0	2.2	0.2	27.0	1.96	2.85	
		1	68.6	2.6	0.3	27.8	2.14	3.00	
		1	69.0	2.3	0.3	27.6	2.09	3.14	
		1	72.3	1.6	0.0	26.4	1.82	2.86	
Average		6	70.4	1.8	0.2	27.0	1.97	3.03	154

[1] This was fat extracted from chicks fed 0.1% cottonseed oil.

[2] Assuming random distribution. The values are approximations on the assumption that all the acids have the same molecular weight.

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